Ribavirin, interferon, and antibody approaches to prophylaxis and therapy for viral hemorrhagic fevers

Thomas P. Monath, MD

US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA

Current Opinion in Infectious Diseases 1990, 3:824–833

The hemorrhagic fever syndrome is caused by at least 15 viruses with single-stranded RNA genomes, which belong to four virus families (Table 1). Despite their diverse etiologies, the viral hemorrhagic fevers share certain clinical and pathophysiologic features, including damage or dysfunction of vascular endothelium, a hemorrhagic diathesis, shock, and varying degrees of hepatic, renal, and central nervous system impairment. To some extent, these similarities may reflect common pathogenetic mechanisms, such as the release of circulating or locally active mediators affecting coagulation, cell metabolism, and capillary permeability. However, accumulating evidence suggests that important differences underlie the pathogenesis of individual viruses. An excellent review by Peters *et al.* (*Curr Top Microbiol Immunol* 1987, 134:5–68) and a recent symposium on the pathogenesis and pathophysiology of these infections [1] contain much information relevant to prophylaxis and therapy. The present review focuses on the antiviral drug ribavirin, which has earned an important place in the management of viral hemorrhagic fevers caused by arenaviruses and bunyaviruses. Mention is also made of the roles of immunotherapy and interferon.

Toxicity, pharmacology, and mechanisms of action of ribavirin

Ribavirin (1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide) was described in 1972 as a purine analogue with broad antiviral activity (Witowski *et al.*, *J Med*

Table 1. Viruses associated with the hemorrhagic fever syndrome and their sensitivity to ribivarin

Family	Genus	Virus	Disease	Ribivarin sensitivity
Arenaviridae	*Arenavirus*	Lassa fever virus	Lassa fever	High
		Junin virus	Argentine hemorrhagic fever	High
		Machupo virus	Bolivian hemorrhagic fever	High
Bunyaviridae	*Hantavirus*	Hantaan virus	HFRS	High
		Seoul virus	HFRS	High
		Puumala virus	Nephropathia epidemica	High
	Nairovirus	CCHF virus	CCHF	High
	Phlebovirus	Rift Valley fever virus	Rift Valley fever	Moderate
Filoviridae	*Filovirus*	Ebola virus	Ebola virus disease	None
		Marburg virus	Marburg virus disease	None
Flaviviridae	*Flavivirus*	Yellow fever virus	Yellow fever	Low
		Dengue virus, types 1–4	Dengue hemorrhagic fever	Low
		Kyasanur Forest disease virus	Kyasanur Forest disease	Unknown
		Omsk hemorrhagic fever virus	Omsk hemorrhagic fever	Unknown

Abbreviations

CCHF—Congo-Crimean hemorrhagic fever; **HFRS**—hemorrhagic fever with renal syndrome.

Chem 1972, 15:1150–1154). Activity has been demonstrated against many DNA and RNA viruses, with a wide separation of cytotoxic and viral inhibitory activities (Smith and Kirkpatrick, eds., *Ribavirin, A Broad-Spectrum Antiviral Agent.* Academic Press, 1980). In clinical trials conducted since 1977, ribavirin has been administered to more than 1000 cancer patients and more than 2000 patients with various viral infections, including influenza, measles, hepatitis A, B, and C, and herpes (Fernandez, in Smith and Kirkpatrick, eds. *Ribavirin, A Broad-Spectrum Antiviral Agent.* Academic Press, 1980, pp 215–232; Smith *et al.*, eds., *Clinical Applications of Ribavirin.* Academic Press, 1984).

The efficacy of ribavirin administered as a small particle aerosol against respiratory syncytial virus was demonstrated in experimentally infected adults (Hall *et al.*, *N Engl J Med* 1983, 308:1443–1447), leading to licensure of the drug for this indication in the United States. The oral formulation is approved for use against influenza, hepatitis, and herpesvirus infections in many countries, but rigorous delineation of efficacy is still required. The drug is still under study in human immunodeficiency virus–infected patients; despite *in vitro* activity against human immunodeficiency virus, ribavirin has not suppressed antigenemia or caused immunologic improvement (Roberts *et al.*, *AIDS* 1990, 4:67–72; Schulof *et al.*, *J AIDS* 1990, 3:485–492). Intravenous ribavirin has been evaluated principally for the treatment of viral hemorrhagic fevers (as described in the following sections), but limited trials have also been reported against herpes zoster (Lorenco *et al.*, *Rev Bras Med* 1977, 33:401–403) and influenza myocarditis (Ray *et al. J Infect Dis* 1989, 159:829–836).

Extensive human use has supported the safety of ribavirin. The most important documented adverse effect in humans is anemia due to inhibition of erythrocyte maturation in bone marrow and to peripheral hemolysis (Canonico, in Hahn, ed. *Antibiotics, Vol VI, Modes and Mechanisms of Microbial Growth Inhibitors.* Springer-Verlag, 1983, pp 160–186). These effects are dose-dependent and completely reversible on cessation of therapy. In a study of human immunodeficiency virus–infected men with lymphadenopathy, Roberts *et al.* (*Clin Pharmacol Ther* 1987, 42:365–373) noted reductions in hematocrit of 0, 4, and 12 points in subjects respectively receiving 600, 1200, and 2400 mg/d for 2 weeks. Patients treated for as long as 24 weeks developed a compensatory reticulocytosis, with stabilization of the anemia after its nadir at 2 to 3 weeks of therapy; the hematocrit rapidly returned to normal after cessation of the drug therapy. A higher incidence of subjective complaints, *ie*, gastrointestinal symptoms (flatulence and nausea), metallic taste, and central nervous system symptoms (headache, insomnia, and fatigue), was noted among treated patients. Although not statistically different from the reactions of placebo controls at daily doses of 1200 mg or less, these reactions were moderately severe at daily doses of 2400 mg, requiring cessation of therapy in some patients.

Although the anemia and other adverse reactions noted previously do not limit use of the drug for treatment of serious viral infections, they assume another dimension in the prophylactic use of ribavirin. The administration of ribavirin to healthy individuals or those exposed to self-limited viral infections must also take into account certain toxic effects of ribavirin described only in experimental animals, including teratogenicity and fetotoxicity (clearly established in rabbits and rodents), testicular atrophy (inconclusive in rodents), and carcinogenicity (inconclusive in rodents). Use of the drug in women of child-bearing age requires that pregnancy be excluded, not only during the course of treatment, but during drug washout, which is prolonged. Prophylaxis (and treatment) of pregnant women assumes its greatest importance in the case of Lassa fever (discussed in a later section). Further studies on the questions of testicular damage (and its reversability) and of carcinogenicity are currently underway in rodents.

The pharmacodynamics of ribavirin in humans have been studied after intravenous (Austin *et al.*, *Antimicrob Agents Chemother* 1983, 5:696–701; Laskin *et al.*, *Clin Pharmacol Ther* 1987, 41:546–555) and oral administration (Roberts *et al.*, *Clin Pharmacol Ther* 1987, 42:365–373). Several points emerge that are relevant to the viral hemorrhagic fevers. After a single intravenous administration of 2400 mg (the loading dose used for treating hemorrhagic fever patients), the mean peak (0.5-hour) serum concentration was 40 μg/mL. In Lassa fever patients treated for 4 days with 1000 mg every 6 hours, the mean level 2.5 hours after the dose was 8 μg/mL. These levels are within the range for inhibition of arena- and bunyaviruses in cell culture (Table 2). The inhibitory effect of ribavirin is highly host-cell dependent, probably because of differences in the ability to phosphorylate the drug to its active form. Vero cells are among the most resistant cell types.

Moreover, plasma levels may not accurately reflect intracellular concentrations of ribavirin, which is a prodrug requiring phosphorylation by cellular enzymes for its antiviral action. The drug is concentrated in erythrocytes in phosphorylated forms, accumulates with repeated dosing, and has a very long elimination half-life (> 2 weeks) corresponding to the life-span of erythrocytes. The long terminal half-life of ribavirin, its entrapment within erythrocytes, and prolonged time required to reach steady-state conditions, are indications for use of a loading dose and frequent schedule of intravenous drug administration when treating life-threatening acute infections.

Only about 25% of the drug is excreted in urine, and the drug is not significantly cleared by hemodialysis (Kramer *et al.*, *Antimicrob Agents Chemother* 1990, 34:489–490). Thus, the dose does not have to be adjusted in patients with renal failure, an important point for patients with some hemorrhagic fevers. Ribavirin is primarily eliminated by metabolic pathways, and high concentrations are found in liver tissue (Ferrara *et al.*, *Antimicrob Agents Chemother* 1981, 19:1042–1049), a critical target organ for many hem-

Table 2. Epidemiologic chacacteristics of bunyviral and arenaviral hemorrhagic fevers and efficacy of various therapeutic approaches

	Arenavirus infections			Bunyavirus infections			
	Lassa fever	Argentine hemorrhagic fever	Bolivian hemorrhagic fever	HFRS		CCHF	Rift valley faver
Epidemiologic characteristics							
Geographic site	West Africa	Buenos Aires and Corboda Province	Eastern Bolivia	Far East (Hantaan virus), Scandanavia (puumala virus)		Africa, Middle East, USSR, Asia	Africa
Reservoir or vector	Rodent	Rodent	Rodent	Rodent	Rodent	Tick	Mosquito
Person-to-person spread	+	Rare	Rare	No	No	++	?0
Estimated annual incidence, *n*	30,000	300–1000	?none	>100,000	>1000	100–200	Thousands in epidemics
Case-to-fatality ratio, %	16	10–15	10–15	5–10	<0.5	30	0.3–0.5
Animal model	Guinea pig, monkey	Guinea pig, monkey	Guinea pig, monkey	Suckling mouse	SCID mouse	Suckling mouse, SCID mouse	Mouse, monkey
Therapeutic approaches							
Ribavirin							
Approximate median effective dose, μg/mL*	20	20	30	≈15		4	80
Effective in animal models	++	++‡	++‡	++	NT	++	++
Effective in clinical trials	++	+	NT	++	NT	+	NT
Interferon							
Sensitive *in vitro*	0	0	0	++	++	++	++
Effective in animal models	0*	0*	0*	NT	NT	NT	++
Effective in clinical trials	NT	Endogenous interferon pathogenic	NT	0	NT	NT	NT
Immune plasma							
Effective in animal models	++	++	++‡	NT	NT	NT	NT
Effective in clinical trials	+/0†	++‡	NT	Not indicated		+/0†	NT

HF—hemorrhagic fever; NT—not tested; SCID—severe combined immunodeficiency disease.
0—not present, not effective; +—occasionally present, efficacy modest or uncertain; ++often present, efficacy proven; +/0—efficacy demonstrated in some studies, but not in others.
*Determined in Pichinde guinea pig model.
†Efficacy dependent on neutralizing antibody titer of plasma
‡Treatment associated with late neurologic syndrome.

orrhagic fever viruses. However, the drug inefficiently crosses the blood-brain barrier, and prolonged administration is required for cerebrospinal fluid concentrations approaching those in plasma (Crumpacker *et al.*, *Lancet* 1986, ii:45–46; Ogle *et al.*, *J Infect Dis* 1989, 159:748–750). Because many hemorrhagic fever viruses are both neurotropic and viscerotropic, an encephalitic component of the disease process may be expressed after successful ribavirin treatment of the acute visceral (hemorrhagic) stage of infection.

The action mechanisms of ribavirin remain controvertial: several mechanisms have been postulated that are

not mutually exclusive. Ribavirin is a guanosine analogue and competitively inhibits intracellular pools of guanosine triphosphate required for viral nucleic acid synthesis. A second proposed mechanism involves the formation of absent or abnormal 5′-cap structures of messenger RNA. A third possibility is a direct interference with viral polymerase function and messenger RNA transcription; this effect has been demonstrated for a bunyavirus [2]. Finally, in the case of an arenavirus, ribavirin apeared to induce a posttranslational modification of the nucleoprotein and interference with binding to RNA (Gessner and Lother, *J Virol* 1989, 63:1827–1832).

Treatment of specific diseases

Ribavirin has a prominent place in the prophylaxis of, and therapy for, hemorrhagic fever viruses belonging to the families Arenaviridae and Bunyaviridae [3]. *In vitro* sensitivity is predictive of the antiviral effects *in vivo* (Table 2). Comparison of the concentrations required for 50% inhibition of virus yields in Vero cells reveals a high sensitivity of arenaviruses and Congo-Crimean hemorraghic fever (CCHF) virus (1 to 20 μg/mL) and lower sensitivity of phleboviruses, *eg*, Rift Valley fever and sandfly fever (40 to 80 μg/mL) [4]. Ribavirin is not effective against the filoviruses, Ebola, and Marburg virus diseases. Flaviviruses require concentrations (250 to 400 μg/mL) that are generally not achievable with nontoxic doses *in vivo*.

Arenavirus diseases

Lassa fever

Lassa fever is an acute infection characterized by fever, headache, myalgia, pharyngitis, vomiting, a relatively mild hemorrhagic diathesis, a capillary leak syndrome with facial edema, adult respiratory distress syndrome, shock, and multiple organ failure (McCormick *et al.*, *J Infect Dis* 1987, 155:445–455). Eighth nerve deafness is an important complication in surviving patients.

Lassa fever is endemic in West Africa, where an estimated 300,000 human infections, 30,000 clinical cases, and 5000 deaths occur annually (McCormick *et al.*, *J Infect Dis* 1987, 155:437–444). The virus is transmitted to humans by the commensal rodent, *Mastomys natalensis* (Monath *et al.*, *Science* 1974, 185:263–265). Infection rates in some villages may exceed 12% per year, and more than a third of all deaths on medical wards of hospitals in endemic areas are due to the disease, with a case-fatality rate in hospitals of 16%. A number of nosocomial outbreaks have been described in which hospital staff and patients became infected by contact or possibly aerosol exposure to an index case (Monath *et al.*, *Am J Trop Med Hyg* 1972, 22:773–779). Fortunately, such events have been unusual, and the risk of infection among hospital workers in West Africa does not exceed that in the indigenous population (Helmick *et al.*, *Lancet* 1986, ii:1202–1205).

Simple barrier nursing procedures appear to be effective in preventing nosocomial infections (*MMWR* 1988, 37 [suppl 3]:1–16). At least 20 patients with Lassa fever imported into the United States [5], Canada, Europe, Israel, and Japan have been medically treated or hospitalized before the diagnosis was suspected, without secondary spread of the virus to more than 1000 contacts. Nevertheless, the occurrence of occasional nosocomial outbreaks in Africa, the possible person-to-person spread under conditions of close contact in familial groups (Keenlyside *et al.*, *Am J Trop Med Hyg* 1983, 32:829–837), and the appearance of unsuspected cases in travelers will continue to provide opportunities for antiviral prophylaxis [5].

The disease in experimentally infected rhesus monkeys closely resembles that in humans. Ribavirin treatment initiated as late as 5 days after virus prevented lethal infection, reduced cell injury as reflected by serum transaminase levels, and abrogated viremia (Jahrling *et al.*, *J Infect Dis* 1980, 141:580–589), inspiring clinical trials in West Africa of oral and intravenous ribavirin, as well as of convalescent plasma (McCormick *et al.*, *N Engl J Med* 1986, 314:20–26). These trials were open-label trials in which mortality in treated patients was compared with that in historical controls; moreover, a retrospective reanalysis of data on a subset of patients was performed after fatal-outcome predictors were identified (aspartate aminotransferase levels, > 150 IU/L; or viremia, > 3.6 $\log_{10}$; median tissue culture infective dose, 50/mL). Despite these shortcomings in design and analysis, a convincing case was made for efficacy of ribavirin. The best survival was observed in patients treated on or before the sixth day of illness with intravenous drug. In the subset of patients with the worst prognosis (viremia, > 3.6 logs), the case-fatality rate was 9% in patients treated with intravenous ribavirin and 20% in those given the drug orally, as compared with 75% in untreated (historical) controls.

Convalescent plasma was not efficacious. However, this finding may have been due to the absence or low titers of neutralizing antibodies in convalescent plasmas used in this study. Neutralizing antibodies may not be detectable until months after infection, and careful selection of convalescent plasma is required to yield material suitable for passive immunotherapy (Jahrling *et al.*, *Trans R Soc Trop Med Hyg* 1985, 79:380–384). Potent plasma given prophylactically or within a few days after virus protects experimental animals from lethal infection (Jahrling *et al.*, *J Infect Dis* 1984, 149:420–427). Combined use of ribavirin and potent plasma rescued monkeys in which treatment was begun as late as 10 days after infection (when either treatment alone was unsuccessful). These results may be relevant to the clinical situation, especially to antibody treatment of pregnant women (in whom ribavirin is contraindicated) and to combined ribavirin-antibody treatment of patients who present 7 days or more after onset, when use of the antiviral drug alone is less effective. A large volume of potent plasma from recovered Liberian patients has recently been fractionated to yield IgG

for intravenous use and awaits clinical testing (Peters, personal communication). A potential problem with the use of immunotherapy is the antigenic diversity of Lassa virus strains and the possibility that antibodies from Liberia will prove less potent against other geographic strains of the virus, as has been shown in animals by Jahrling and Peters (*Infect Immun* 1984, 44:528–533).

Present recommendations (*MMWR* 1988, 37 [suppl 3]:1–16) [4] suggest that large doses of oral ribavirin be given to contacts who have unprotected contact with a patient's body fluids or excretions (including exposure to blood, kissing, sexual intercourse, sharing food or utensils, and other forms of contact). The definition of what constitutes high risk and the recommended dose of oral ribavirin for postexposure prophylaxis have been called into question [5]. Although few data on the efficacy of dosing schedules are available, the Centers for Disease Control recommendations for prophylactic treatment call for administration of 2 to 2.4 g daily, a level associated with significant anemia and other side effects. A more modest daily dose of ribavirin may be appropriate for prophylaxis, because initial trials in Africa of oral ribavirin for *treatment* of Lassa fever showed that 1.0 g daily for 10 days significantly reduced mortality (McCormick *et al.*, *N Engl J Med* 1986, 314:20–26). Given the apparent low risk of contagion, close medical surveillance and early treatment of compatible febrile illness with intravenous ribavirin may be an acceptable strategy for all but those with the highest risk of infection, *eg*, those with a needle-stick injury or direct blood contact with mucous membranes or broken skin.

Two final points relating to ribavirin therapy should be mentioned. First, Lassa fever is an especially severe infection in pregnant women [6]. Fetal loss is 92% during the first and second trimesters and 75% in the third, and the risk of maternal death is significantly higher in the third trimester than earlier in pregnancy or in nonpregnant women. Since termination of pregnancy and uterine evacuation significantly improve survival, this course of action is recommended prior to therapy with ribavirin. Second, antiviral treatment of experimental and human Lassa fever has not been associated with late encephalitis or encephalopathy, as described in the other arenaviral hemorrhagic fevers. This observation may reflect a lower neurotropism of Lassa virus.

Argentine and Bolivian hemorrhagic fevers

The Argentine and Bolivian hemorrhagic fevers, which are rodent-borne South American fevers, share clinical features with Lassa fever but have more prominent neurologic symptoms and hemorrhagic manifestations. The case-fatality ratio in both diseases is approximately 10% to 15%, with deaths occurring from irreversible shock during the second week of illness. Argentine hemorrhagic fever, caused by Junin virus, occurs in an endemic, localized region of Buenos Aires and Córdoba Provinces, with an annual incidence of several hundred cases and cyclic epidemics with up to several thousand cases. A live, attenuated vaccine now undergoing phase III trials in Argentina is expected to reduce dramatically the need for therapeutic interventions. Bolivian hemorrhagic fever, caused by Machupo virus, was responsible for dramatic epidemics in eastern Bolivia in the early 1960s, but no cases have been documented in recent years. Since neither disease is readily contagious (viremia levels in severe cases of Argentine hemorrhagic fever are 100-fold lower than those in Lassa fever), postexposure prophylaxis with antiviral drugs or antibody is appropriate only in special circumstances of direct blood or virus exposures (needle-stick injury, laboratory accidents.)

Nonhuman primates provide useful models for studying the pathogenesis and treatment of the South American hemorrhagic fevers. Ribavirin was first evaluated in rhesus monkeys infected with Machupo virus (Stephen, in Smith and Kirkpatrick, eds. *Ribavirin, a Broad-Spectrum Antiviral Agent.* Academic Press, 1980, pp 169–183) and subsequently in marmosets and rhesus monkeys infected with Junin virus (Weissenbacher *et al.*, *J Med Virol* 1986, 20:261–267; McKee *et al.*, *Antimicrob Agents Chemother* 1988, 32:1304–1309). Ribavirin administered prophylactically to Junin-infected macaques was completely protective, and viremia was not detectable; antibody responses were delayed, probably reflecting the profound suppression of virus replication rather than drug-induced immunomodulation. When ribavirin was initiated at the time of onset of fever or viremia (postinfection days 4 to 7), animals were rescued from the acute hemorrhagic phase of illness and had blunted viremias, but approximately 75% succumbed to late-onset encephalitis. Virus is readily recoverable from the central nervous system of untreated animals that die of viscerotropic, hemorrhagic infection but has very rarely been demonstrated in monkeys dying of late neurologic syndrome. The exact time of viral neuroinvasion has not been established, but neuroinvasion probably occurs as viremia levels escalate during the second week after infection. Delay in treatment with ribavirin, which does not readily enter the central nervous system, allows clinical expression of encephalitis. Treatment of monkeys and guinea pigs with immune plasma also induces late neurologic syndrome.

These events have correlates in human medicine. Convalescent plasma containing a high titer of neutralizing antibodies is highly effective in reducing mortality (to < 1%) if given within 8 days of onset and constitutes standard therapy for Argentine hemorrhagic fever (Maiztegui *et al.*, *Lancet* 1979, ii:1216–1217; Enria *et al.*, *Lancet* 1984, ii:255–256). In the South American hemorrhagic fevers, in contrast to Lassa fever, neutralizing antibodies appear relatively early and, with complement, play an important role in viral clearance and recovery from infection. However, antibody therapy is associated with the appearance of late neurologic syndrome several weeks after recovery in 8.6% of patients, whereas this complication has not been observed in untreated survivors (Enria *et al.*, *Med Microbiol Im-*

munol 1986, 175:173–176). Late neurologic syndrome differs from the severe neurologic syndrome seen during the acute phase and is generally characterized by fever and benign cerebellar signs. The pathogenesis of both the acute and late neurologic syndromes is presently uncertain, and virus is only rarely detectable in brain tissue of patients dying in the acute phase of illness. Nevertheless, several lines of evidence point to persistence of viral antigen in the central nervous system and an immunopathologic basis for late neurologic syndrome: 1) the finding of enhanced serum antibody and cellular immune responses, 2) the presence of antibody in cerebrospinal fluid, and 3) high levels of 2',5'-oligoadenylate synthetase in peripheral blood monocytes (Ferbus *et al.*, *J Infect Dis* 1988, 157:1061–1064).

Only limited experience has been accumulated in ribavirin treatment of humans with Argentine hemorrhagic fever. An open-label clinical trial was performed in Argentina in 1986. Six patients with confirmed Argentine hemorrhagic fever of more than 8 days' evolution were given full-dose intravenous ribavirin for 10 days (Enria *et al.*, *Antiviral Res* 1987, 7:353–359). Three of 6 patients died, but the time to death was prolonged and both the disappearance of viremia and a decline in serum interferon levels were attributed to treatment. Although virus is recoverable from blood and liver in more than 80% of untreated patients at the time of death, no virus was detectable in tissues from the ribavirin-treated patients. Although none of the ribavirin-treated survivors developed late neurologic syndrome, the number of cases is insufficient to draw firm conclusions on the possibility that ribavirin may predispose humans as well as nonhuman primates to this syndrome.

A placebo-controlled, double-blind, phase II trial of intravenous ribavirin (34 mg/kg body weight loading, 16 mg/kg every 6 hours for 4 days, 8 mg/kg every 8 hours for 6 days) was subsequently initiated. A total of 18 patients entered the study, all with at least 9 days of illness prior to entry (beyond the point at which therapy with immune plasma is efficacious). Although not statistically significant, mortality was lower in the ribavirin-treated group. No treatment effect on viremia or interferon levels was observed in this study (McKee and Maiztegui, Personal communication).

Arenaviruses, including Lassa fever and the South American hemorrhagic fevers, are resistant to the action of interferon alpha , despite its induction of high levels of antiviral enzymes (2'5'-oligoadenylate synthetase). Whether interferon itself plays a pathogenic role is unclear. In a guinea pig model of hemorrhagic fever, treatment with interferon or an interferon inducer had neither a beneficial nor an adverse effect (Lucia *et al.*, *Antiviral Res* 1989, 12:279–292). However, very high levels of endogenous interferon are seen in human patients with Argentine hemorrhagic fever and correlate with symptomatology (fever, myalgia, neurologic signs) and severity of illness (Levis *et al.*, *J Interferon Res* 1985, 5:383–389). Moreover, effective treatment with plasma or administration of ribavirin reduces interferonemia. Administration of exogenous interferon to patients with arenaviral infections would thus appear to be contraindicated.

Bunyavirus diseases

Hemorrhagic fever with renal syndrome

Hemorrhagic fever with renal syndrome (HFRS) gained prominence in Western medicine during the Korean conflict, when more than 3000 United Nations soldiers were affected with what was then called Korean hemorrhagic fever. The disease remains a significant military medical problem [6]. The etiologic agent, Hantaan virus, which was first isolated in 1976, is now known to be the prototype of a genus (*Hantavirus*) whose members have worldwide distribution and considerable medical importance (Lee, *Prog Med Virol* 1982, 28:96–113). In Asia and the USSR, Hantaan virus, transmitted to humans in rural environments by field mice, and Seoul virus, transmitted by rats in urban areas, account for more than 100,000 cases annually. The disease associated with Hantaan virus is severe, with a case-fatality rate of 5% to 10%, whereas the urban form is milder (case-fatality, ≤ 1%). In Scandanavia and parts of western Europe, a related virus (Puumala) transmitted by voles causes hundreds to thousands of cases of a generally benign form (case-fatality, < 0.5%), referred to as *nephropathia epidemica*. Seoul-like viruses have been widely disseminated by domestic rats. Human infections are documented in urban residents in the United States (Childs, *Am J Epidemiol* 1988, 127:875–878) and may be responsible for chronic hypertensive renal disease. Outbreaks have also been associated with infec d laboratory rats.

The full clinical expression of Hantaan virus disease is characterized by five sequential phases of illness (McKee *et al.*, in Belshe, ed. *Human Virology*. PSG Publ. Co. 1990, pp. 1866–1879). The *febrile phase* (lasting 3 to 7 days) is characterized by fever, myalgia, gastrointestinal symptoms, renal pain and tenderness, and signs of vascular dysregulation. This phase is followed by a *hypotensive phase* (lasting several hours to 3 days), with capillary leak syndrome, hemoconcentration, hypotension, shock, proteinuria, and hemorrhagic phenomena; approximately one third of the deaths occur during this phase. The *oliguric phase*, lasting 3 to 7 days, is characterized by renal failure, hypertension, severe hemorrhagic manifestations, metabolic, and electrolyte derangements, and pulmonary edema, and accounts for more than 50% of the deaths. A *diuretic phase* follows, with recovery from renal tubular necrosis. *Convalescence* may be prolonged, often requiring months for full return to health. No animal model of this disease process has been discovered.

The pathogenesis of HFRS is relevant to antiviral therapy. The long incubation period (often 1 month or more), the appearance of clinical disease in the face of a well-established immune response, and evidence for both infectious virus and immune complexes in circulation and in vascular deposits suggest an immunopatho-

logic basis for the disease. Activation of complement, kinin and renin systems, and the occurrence of disseminated intravascular coagulation provide clues to the complex mechanisms underlying vascular damage in HFRS [7]. The secondary and tertiary pathogenetic pathways in this disease underscore the difficulty in treating clinically established disease by interrupting the underlying primary process of virus replication with an antiviral drug; moreover, the use of exogenous antibody would be counterproductive.

In this light, it is gratifying to learn that ribavirin is clinically useful in HFRS (Huggins *et al.*, *Antiviral Res* 1988, 9:183). Preclinical evaluation of ribavirin had shown high *in vitro* sensitivity of Hantaan virus and a positive treatment effect in suckling mice (Huggins *et al.*, *J Infect Dis* 1986, 153:489–497) and severe combined immunodeficiency disease mice (Zhang and Huggins, *Antiviral Res* 1990, [suppl I]:120). This evidence led to a technically difficult, placebo-controlled, double-blind clinical trial in central China between 1985 and 1987. The team of American and Chinese investigators demonstrated significant efficacy of ribavirin initiated on or before the sixth day of illness (Huggins *et al.*, submitted to *J Infect Dis*; Zhang *et al.*, *Clin J Clin Pharmacol* 1988, 4:1–8 [in Chinese]; Zhang *et al.*, *Clin Pharm Bull* 1987, 22:246–248 [in Chinese]). At this point in the infection, nearly all patients' conditions may be specifically diagnosed by a rapid serologic test (IgM enzyme-linked immunosorbent assay).

Ribavirin was administered intravenously in a dose corresponding to that used in Lassa and Argenitine hemorrhagic fever patients (loading dose of 33 mg/kg, followed by 16 mg/kg every 6 hours for 4 days, then 8 mg/kg every 8 hours for 6 days). Treated patients had a reduction in mortality (2.4% vs 8.6% in placebo-receiving controls; $P = 0.01$); when these figures were corrected for baseline variables predictive of fatal outcome (total serum protein and aspartate aminotransferase levels), a seven-fold reduction was found in risk of death in the treated group. Statistically significant reductions were also demonstrated in the frequency of oliguria, in serum creatinine levels, in the risk of hemorrhage, and in the duration of hypertension. The diuretic phase was significantly foreshortened in treated patients. The only adverse effect related to treatment was the occurrence of anemia, which was not of sufficient magnitude to require interruption of therapy. Ribavirin treatment also decreased the frequency and titer of detectable viremia, as compared with these parameters in control subjects given placebo (Yang *et al.*, *Chung Hua I Hsueh Tsa Chih* 1989, 69:621–625 [in Chinese]).

A placebo-controlled, blinded trial of recombinant interferon alpha was conducted in Chinese patients with moderate or severe HFRS (Gui *et al.*, *J Infect Dis* 1987, 155:1047–1051). No clear benefit was observed in most parameters measured, although bleeding manifestations and proteinuria were significantly less in the treated patients. Mortality was 16% in treated and placebo groups. It is possible that higher doses or earlier institution of interferon treatment, or a combination of interferon and ribavirin might show a more favorable response.

Congo-Crimean hemorrhagic fever

This tick-borne virus belonging to the Nairovirus genus of the family Bunyaviridae is widely distributed in Africa, eastern Europe, the Middle East, the USSR, and Asia. A comprehensive review of the epidemiology of CCHF is provided by Watts *et al.* (in Monath, ed. *The Arboviruses: Epidemiology and Ecology*, vol. II. CRC Press, 1988, pp 117–222). The disease is sporadic, acquired by tick-bite and occupational exposure to blood of infected livestock, and of low incidence. However, an important epidemiologic feature of CCHF is the occurrence of nosocomial outbreaks. In these situations, an index case patient admitted to hospital with hemorrhage undergoes surgery or rescuscitative measures, exposing medical staff to blood. The etiology only becomes apparent with the occurrence of secondary cases among contacts. A mouse-brain vaccine has been developed and extensively applied in Bulgaria, which has the highest incidence of the disease.

Congo-Crimean hemorrhagic fever is a severe infection with a very short incubation period (mean, 3 to 5 days, depending on route of infection), prominent hemorrhage, DIC, shock, multiple organ failure, and a case-fatality of 30%. Patients with fatal cases are viremic until death and have absent or delayed antibody responses [8], suggesting a role for passive antibody treatment and antiviral therapy. Moreover, laboratory parameters, including elevated serum transaminase levels, high total leukocyte count, and various indicators of DIC are predictive of fatal outcome [9] and provide a means for assessing drug efficacy. Early, rapid diagnosis may be achieved by detecting circulating antigen by immunoassay or reverse hemagglutination.

Congo-Crimean hemorrhagic fever virus infectivity is inhibited *in vitro* by low concentrations of ribavirin [3]. The efficacy of ribavirin against CCHF virus *in vivo* has been demonstrated in suckling mice, the only susceptible animal model (Berezina *et al.*, *Vopr Virusol* 1983, 5:627–629; Tignor, Personal communication). Significant protection was achieved with doses of 100 mg/kg administered as late as 48 hours after infection and with lower doses (25 mg/kg) in a prophylactic model.

Experience with ribavirin treatment of human patients with CCHF is very limited. In a nosocomial outbreak in South Africa in 1984, the drug was administered prophylactically to six of nine case-contacts who had sustained direct blood exposure (*eg*, needle-pricks); three individuals also received a short course of interferon. Ribavirin (a minimum of 10 mg/kg every 8 hours) was administered by the intravenous route for one incubation period (8 days) after exposure (Van de Wal *et al.*, *S Afr Med J* 1985, 68:729–732). One of the six treated contacts developed a mild case of CCHF; she had not received interferon. All three untreated contacts developed severe CCHF.

In succeeding years, 12 patients with established CCHF were treated in open-label trials in South Africa (Swanepoel *et al.*, *Proceedings of the Eighth International Congress on Virology* 1990, Abstr P70-3, p 430). Three of 5 patients in whom therapy was initiated late (≥5 days after onset) died (60%), as compared with 0 of 7 patients treated earlier. Of the 7 survivors, 2 had the clinical laboratory markers associated with greater than 90% mortality in untreated (historical) controls.

The role of immunotherapy in patient management remains uncertain. The absence of detectable endogenous neutralizing antibodies and the persistence of viremia in most fatal cases suggests that passive administration of antibody may be beneficial. However, clinical results have been variable. As in Lassa fever, neutralizing antibody levels in convalescent plasmas are generally low, and units used for therapy have generally not been selected for potency. Even when plasmas with moderate titers were used, only transient improvement in clinical status was noted (Van Eeden *et al.*, *S Afr Med J* 1985, 68:718–721), without a clear effect on viremias. Bulgarian workers nevertheless believe that intravenous administration of hyperimmune globulin prepared from vaccinated individuals is effective (Vasilenko *et al.*, *Lancet* 1990, i:791–792).

High doses of human leukocyte interferon administered to South African patients prophylactically and therapeutically (together with plasma) resulted in marked side effects, and therapy was discontinued. No clear picture of efficacy emerged (Van Eeden *et al.*, *S Afr Med J* 1985, 68:718–721).

Rift Valley fever

This disease affecting both humans and domestic livestock is distributed in sub-Saharan Africa and is caused by a mosquito-borne virus belonging to the *Phlebovirus* genus, family Bunyaviridae. On one occasion (1977 to 1978), the virus was exported beyond its natural range to the Nile Delta of Egypt, causing an explosive outbreak (Meegan, *Trans R Soc Trop Med Hyg* 1979, 73:618–623). The possibility of similar occurrences, particularly with spread to the Middle East, has long concerned public health authorities. Recent outbreaks have occurred in Mauritania (1987) and Madagascar (1990). The typical human disease is characterized by a nonspecific but severe febrile syndrome of 4 to 7 days' duration; however, approximately 1% of infections result in severe or complicated illness with hemorrhagic fever, encephalitis, or retinitis. Mortality rates in affected populations have ranged between 0.3% and 0.5%. The case-fatality rate in Egyptian hospitals was 11% to 14%, accounted for mainly by patients with hemorrhage, severe liver dysfunction, and shock. Viremia titers are high, and rapid, early diagnosis of up to one third of human cases may be achieved by enzyme-linked immunosorbent assay detection of antigen in serum or by nucleic acid hybridization; application of the polymerase chain reaction has not yet been reported.

Rift Valley fever virus is susceptible to ribavirin *in vitro*, but higher concentrations are required for its inhibition than for inhibiting Hantaan or CCHF viruses [2,3]. Nevertheless, extensive experience has repeatedly demonstrated efficacy of the drug in mouse and hamster models of Rift Valley fever (Stephen *et al.*, in Smith and Kirkpatrick, eds. *Ribavirin, a Broad-Spectrum Antiviral Agent.* Academic Press, 1980, pp 169–183; Peters *et al.*, *Antiviral Res* 1986, 6:285–297) and related phleboviruses (Sidwell *et al.*, *Antimicrob Agents Chemother* 1988, 32:331–336). Significant protection against Rift Valley fever can be demonstrated by treatment schedules delayed up to 36 hours after infection. Animals challenged with virus intracerebrally or those given suboptimal treatment and challenged with virus by the parenteral route are rescued from lethal Rift Valley fever hepatitis, only to die later of encephalitis. A similar alteration of pathogenesis is seen in some arenavirus infections and illustrates the relative inability of ribavirin to cross the blood-brain barrier. The efficacy of ribavirin was enhanced five-fold by incorporation into liposomes, which apparently home to sites of virus replication in the liver (Kende *et al.*, *Antimicrob Agents Chemother* 1985, 27:903–907).

Rhesus monkeys inoculated with a high dose of Rift Valley fever virus provide a realistic model of the human disease [10]. Monkeys treated with ribavirin had absent or significantly diminished viremias, as compared with controls (Peters *et al.*, *Antiviral Res* 1986, 6:285–297). Recent studies using a Rift Valley fever monkey model in which 38% of the infected animals sickened and 12.5% died showed that ribavirin given prophylactically resulted in complete protection from disease and significantly reduced viremia and chemical evidence of hepatic dysfunction (Huggins *et al.*, *Antiviral Res* 1990, [suppl 1], 121). Because Rift Valley fever virus readily invades the central nervous systems of monkeys, it will be important to learn whether ribavirin administered on a therapeutic schedule (after establishment of viremia) will be associated with late encephalitis.

Rift Valley fever virus is sensitive to interferon *in vitro*, and interferon appears to play an important role in recovery from acute infection [11]. Relatively low doses of exogenous interferon alpha protected monkeys against viremia, hepatic dysfunction, and coagulopathy [12], and interferon inducers are effective in rodent models. Synergism in rodent models between ribavirin and immunomodulators, including poly (ICLC) (Kende *et al.*, *Adv Biosci* 1988, 68:51–63), suggests interesting approaches to prophylaxis and therapy in humans.

No human trials of ribavirin, interferon, or combined therapy have yet been conducted. Clinical studies would certainly be appropriate in the event of a future epidemic. To better define the appropriate dose and schedule of prophylactic and therapeutic approaches, the sandfly fever clinical model will prove useful. This self-limited febrile disease, caused by a phlebovirus related to Rift Valley fever virus, has long been used

for experimental infection of normal human subjects (Friman *et al.*, *Acta Med Scand* 1985, 217:353–361). A double-blind, placebo-controlled trial of oral ribavirin was conducted for the prophylaxis of this viral disease (MacDonald *et al.*, Paper presented at the 86th Annual Meeting of the American Society of Microbiology Washington, DC, 1986). Ribavirin was given orally at a dose of 400 mg every 8 hours beginning 24 hours before infection and continuing for 8 days. None of six treated patients and four of six placebo recipients developed typical sandfly fever (fever and a constellation of typical symptoms, including leukopenia, interferonemia, antigenemia).

Flavivirus diseases

Flaviviral hemorrhagic fevers, including yellow fever, Kyasanur Forest disease, and dengue hemorrhagic fever, are possible target diseases for antiviral therapy. As noted previously, the therapeutic index of ribavirin for flaviviruses is significantly lower than that for arena- and bunyaviruses; no antiviral effect has been demonstrated in rodent flavivirus models. Prophylactic administration of ribavirin to monkeys failed to abrogate dengue viremia [13] or to influence the course of yellow fever infection (Peters *et al.*, *Proceedings of the International Symposium on Yellow Fever and Dengue.* Oswaldo Cruz Inst., 1990, in press). Given the need to achieve very high concentrations for an antiviral effect, future research might focus on delivery of ribavirin to a specific target organ (*eg*, the liver in yellow fever by means of liposomes) or to specific infected cells.

Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

1. •• COSGRIFF TM, ED.: **International symposium on hemostatic impairment associated with hemorrhagic fever viruses.** *Rev Infect Dis* 1989, 11(suppl 4):S669–S897.

Compendium of reviews and definitive papers on the molecular biology, epidemiology, pathogenesis, animal models, clinical aspects, and treatment of the viral hemorrhagic fevers. The focus is on disturbances of vascular endothelium and mechanisms underlying coagulation defects.

2. • CASSIDY LF, PATTERSON JL: **Mechanism of La Crosse virus inhibition by ribavirin.** *Antimicrob Agents Chemother* 1989, 33:2009–2011.

This paper provides the first direct evidence of ribavirin's mechanism of action for a member of the family Bunyaviridae Low concentrations of ribavirin have a direct inhibitory effect on the bunyaviral RNA-dependent RNA polymerase, thereby suppressing messenger RNA transcription.

3. •• HUGGINS JW: **Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug.** *Rev Infect Dis* 1989, 11(suppl 4):S750–S761.

Comprehensive review of the mechanisms of action, toxicity, *in vitro* activity, and efficacy of ribavirin in animal models of the viral hemorrhagic fevers.

4. • WATTS DM, USSERY MA, NASH D, PETERS CJ: **Inhibition of Crimean-Congo hemorrhagic fever viral infectivity yields *in vitro* by ribavirin.** *Am J Trop Med Hyg* 1989, 41:581–585.

Congo-Crimean hemorrhagic fever virus has a very high *in vitro* sensitivity to ribavirin, with reduction in virus yields ninefold greater than those with the phlebovirus, Rift Valley fever. The dose of ribavirin required to effect a 50% reduction in virus yields was lower than that reported for Hantaan and Lassa viruses, supporting the concept that ribavirin will be clinically useful in human cases of CCHF.

5. •• HOLMES GP, MCCORMICK JB, TROCK SC, CHASE RA, LEWIS SM, MASON CA, HALL PA, BRAMMER LS, PEREZ-ORONEZ GI, MCDONNELL MK, PAULISSEN JP, SCHONBERGER LB, FISHER-HOCH SP: **Lassa fever in the United States: investigation of a case and new guidelines for management.** *N Engl J Med* 1990, 323:1120–1123.

An American citizen of Nigerian birth acquired Lassa fever while attending his mother's funeral in Nigeria (she and other family members died of the disease) and returned to the United States, where he died. A total of 102 case-contacts were identified, and 7 high-risk contacts were treated prophylactically with ribavirin. Serologic tests failed to indicate infection among the contacts. Risks of person-to-person spread are discussed and an algorithm for surveillance, prophylaxis, and treatment of febrile illness among case-contacts is presented. However, an editorial by Johnson and Monath in the same issue (pp 1139–1141) raises concerns about the definition of high-risk contact for selection of persons for prophylaxis, about the high ribavirin dose recommendations, and about the criteria for initiation of intravenous therapy in case-contacts.

6. • BRUNO P, HASSELL LH, BROWN J, TANNER W, LAU A: **The protean manifestations of hemorrhagic fever with renal syndrome: a retrospective review of 26 cases from Korea.** *Ann Intern Med* 1990, 113:385–391.

The clinical characteristics of cases of HFRS among servicemen admitted to US military hospitals between 1981 and 1986 are reviewed. Patients presented with undifferentiated febrile illness, acute renal failure, or shock syndrome. The difficulty in clinical diagnosis is emphasized. HFRS was suspected initially in only 27% of the cases; a mean of 2.5 clinic visits occurred before the diagnosis was suspected. The case-fatality rate was 8%. These cases emphasize the need for specific, rapid diagnostic tests and the potential difficulties in early institution of antiviral therapy in a disease with protean clinical manifestations.

7. •• LEE M, KIM B-K, KIM S, PARK S, HAN JS, KIM ST, LEE JS: **Coagulopathy in hemorrhagic fever with renal syndrome (Korean hemorrhagic fever).** *Rev Infect Dis* 1989, 11(suppl 4):S877–S883.

Hemorrhagic manifestations are common in patients with HFRS. Pathologic findings in fatal cases include gross hemorrhage in the renal medulla, right atrium of the heart, and anterior pituitary. Cerebral hemorrhage is an important cause of death. Thrombocytopenia, defective platelet function, and disseminated intravascular coagulation play important roles in the coagulopathy of HFRS. Complement activation, circulating immune complexes, and increased plasma kallikrein levels are features of the infection. Whether direct viral injury to endothelium triggers these events or whether endothelial injury is secondary to them is presently unknown. The clinical efficacy of ribavirin suggests that continuing virus replication in the face of an ongoing immune response is central to the pathogenesis of the acute disease process.

8. • SHEPHERD AJ, SWANEPOEL R, LEMAN PA: **Antibody response in Crimean-Congo hemorrhagic fever.** *Rev Infect Dis* 1989, 11(suppl 4):S801–S806.

Most patients with CCHF develop low titers of neutralizing antibodies during convalescence, emphasizing the need to carefully select plasma for passive immunotherapy. Patients who succumb fail to clear their viremias and do not develop detectable antibodies. It is unclear whether this effect is due to a delay in immunologic responsiveness or to the formation of immune complexes.

9. •• SWANEPOEL R, GILL DE, SHEPHERD AJ, LEMAN PA, MYNHARDT JH, HARVEY S: **The clinical pathology of Crimean-Congo hemorrhagic fever.** *Rev Infect Dis* 1989, 11(suppl 4):S794–S800.

Features of 50 cases of CCHF from South Africa are reviewed and related to pathogenesis and clinical management. The incubation period ranged from 2 to 9 days. Among 38 patients with known exposure, infection resulted from tick-bite in 17, contact with blood of

livestock in 14, and nosocomial spread in 7. The case-fatality rate was 30%, with deaths due to multiple-organ failure. Liver necrosis was a prominent feature, and some patients became overtly jaundiced. Severe bleeding diathesis, marked thrombocytopenia, and disseminated intravascular coagulation were features of the disease. Occurrence of several laboratory markers of hepatic dysfunction and coagulopathy during the first 5 days of illness was associated with more than 90% mortality; these markers will be useful in assessing efficacy of antiviral therapy.

10. •• MORRILL JC, JENNINGS GB, JOHNSON AJ, COSGRIFF TM, GIBBS PH, PETERS CJ: **Pathogenesis of Rift Valley fever in rhesus monkeys: role of interferon response.** *Arch Virol* 1990, 110:195–212.

Rhesus monkeys inoculated with Rift Valley fever virus provide a model of the human disease useful for evaluating antiviral drug therapy and interferon. Eighteen percent of infected monkeys developed fatal hemorrhagic fever and prominent hepatic necrosis. Fatally infected animals also had pathologic evidence of encephalitis. A significant correlation was found between fatal outcome and delayed interferon response.

11. •• PETERS CJ, LIU C-T, ANDERSON GW JR, MORRILL JC, JAHRLING PB: **Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted.** *Rev Infect Dis* 1989, 11(suppl 4):S743–S749.

Recovery of the host from infection with the phlebovirus, Rift Valley fever, depends on the actions of interferon and neutralizing antibody, whereas interferon may be pathogenic in arenavirus infections and cellular immunity, not antibody, is responsible for arenavirus clearance. Direct viral cytolysis underlies injury in Rift Valley fever, whereas Lassa fever pathogenesis may be indirect, involving soluble mediators. Implications for therapy are discussed.

12. •• MORRILL JC, JENNINGS GB, COSGRIFF TM, GIBBS PH, PETERS CJ: **Prevention of Rift Valley fever in rhesus monkeys with interferon-alpha.** *Rev Infect Dis* 1989, 11(suppl 4):S815–S825.

Modest doses of 5×10^4 U/kg of human leukocyte or recombinant interferon alpha given 24 hours before Rift Valley fever virus and then daily for 5 days prevented illness and markedly reduced hepatic and hemostatic dysfunction and viremia. A dose of 5×10^5 U/kg was successful when given 6 hours after virus challenge. Interferon (or interferon inducers) may be useful for prophylaxis of Rift Valley fever infection or possibly for therapy initiated very early in the course of human illness.

13. • MALINOSKI FJ, HASTY SE, USSERY MA, DALRYMPLE JM: **Prophylactic ribavirin treatment of dengue type 1 infection in rhesus monkeys.** *Antiviral Res.* 1990, 13:139–150.

Rhesus monkeys inoculated with dengue type 1 developed low-titered viremias lasting 5 to 6 days, but no illness or clinical laboratory perturbations. Animals were treated with intramuscular ribavirin (10 mg/kg every 8 hours for 10 days beginning 24 hours before virus inoculation; hematologic toxicity was observed, indicating that ribavirin was administered near maximum tolerated dose. No effect was noted on the height or duration of dengue viremia.